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DIFFERENTIAL EFFECT OF MARROW ADIPOSITY AND VISCERAL AND SUBCUTANEOUS FAT ON CARDIOVASCULAR RISK IN YOUNG, HEALTHY ADULTS

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Abstract

Background—Adipose tissue is an endocrine organ that influences many metabolic processes and accumulates in different depots, including the bone marrow. While the negative associations between visceral fat (VF) or subcutaneous fat (SF) and cardiovascular disease (CVD) risks are well known, the relation between marrow fat (MF) and metabolic risk is unexplored.

Objectives—We examined the relations between these three fat depots and whether CVD risks are associated to marrow adiposity.

Design—observational cross sectional study

Subjects and Methods—Computed tomography was used to measure VF, SF and MF depots in 131 healthy young adults (60 females, 71 males; 16-25 years of age). Weight, body mass index (BMI), waist and hip circumferences, blood pressure (BP), carotid intima media thickness (CIMT) and serum levels of lipids, glucose and insulin were also measured.

Results—Regardless of gender, MF was not associated to values of VF or SF, anthropometric measures, or lipid or carbohydrate serum levels (P >0.05 for all). In contrast, VF was associated to SF (r's= 0.74 for females, 0.78 for males; both P's <0.0001) and these depots were related to anthropometric parameters (r's between .69 and .87; all P's <0.0001) and to most measures of lipids, glucose or insulin (r's between .25 and .62).

Conclusions—Marrow adiposity in young men and women is independent of visceral and subcutaneous fat, and is not associated to CVD risk. These findings do not support the concept that marrow adiposity is involved in the comorbidities related to fat accumulation in other compartments.

Keywords

marrow fat; visceral fat; subcutaneous fat; cardiovascular risk; computed tomography

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INTRODUCTION

Fat tissue is a complex endocrine organ, secreting multiple hormones, influencing many metabolic processes, and amassing at various sites, including the subcutaneous and intra-abdominal tissues and the marrow cavity of bones. In excess, it gives rise to several comorbidities (1). A wealth of epidemiologic data links a growing number of negative health outcomes, such as hypertension, insulin resistance, type 2 diabetes, dyslipidemia, the metabolic syndrome, and CVD risk to the progressive accumulation of fat. Despite differences in histology, physiology, and gene expression profile among fat depots, it is generally believed that excessive fat accumulation at any site has similar negative health connotations (2-5). For example, both intra-abdominal and subcutaneous fat depots are related to insulin resistance and dyslipidemia, albeit to different degrees (6-10).

We recently found an inverse relation between fat in the marrow cavity and the amount of bone in the skeleton of young healthy subjects. Unexpectedly, there were no relations between measures of marrow adiposity with weight, BMI or total body fat as measured by dual energy x-ray absorptiometry (DXA) (11). These findings suggest that marrow fat may be an independent fat compartment with potentially different metabolic functions; consistent with studies in animal models reporting no relation between body size or insulin secretion and marrow adiposity (12). However, the possible association between marrow fat and blood pressure (BP), measures of carotid wall thickness and carbohydrate or lipid metabolism in humans is largely unexplored, and no previous study has assessed the relation between marrow adiposity and CVD risk factors. We examined the relations between marrow fat and subcutaneous and visceral adiposity using computed tomography (CT) and between marrow fat and BP, carotid wall thickness, fasting levels of insulin, glucose and lipids in healthy young males and females.

SUBJECTS AND METHODS

Subjects

The participants were 131 healthy young adults (60 females, 71 males; 16-24.9 years of age) who were recruited from schools and colleges in the Los Angeles area. The investigational protocol was approved by the hospital institutional review board and informed consent was obtained. Candidates for this study were excluded if they had a diagnosis of any underlying disease or chronic illness, if they had been ill for >2 weeks during the previous 6 months, if they had been admitted to the hospital at any time during the previous 3 years, or if they were taking any medications regularly. Females who were pregnant were also excluded. All potential candidates underwent a physical examination by a pediatric endocrinologist and only those who had achieved sexual maturity (Tanner stage V of sexual development) were included (13). Thereafter, height, weight, BP, body mass index (BMI), waist and hip circumferences and the waist-to-hip ratio were determined. The waist-to-hip ratio was calculated as a surrogate of central adipose distribution.

Carotid Ultrasonography

All studies were acquired by the same specialized ultrasound (US) technologist using the Acuson Sequoia 512 mainframe (Acuson Corp., California, USA) equipment and a high-resolution 15L8 MHz linear-array transducer following a predetermined standardized scanning protocol. The patients were studied in the supine position with the head turned slightly toward the side examined. Images of the arterial wall were obtained from the posterior walls of both common carotids one centimeter below the carotid bulb (bifurcation) during three complete and independent cardiac cycles. An automated computerized edge detection software package developed by Siemens (Siemens Medical Solutions USA, Pennsylvania, USA) was used to determine carotid intima-media thickness (CIMT) values in the frames of each cycle depicting the narrowest and widest vessel diameters. The mean measure obtained from three independent cardiac cycles at the maximum and minimum arterial diameters in both carotids was used for analysis. All examinations were digitally stored and analyzed by the same researcher. The intraclass correlations for CIMT determinations obtained in three different cardiac cycles are between .92-.98 (14).

Blood Pressure

Readings of BP were taken after a 10-minute rest using a standard mercury sphygmomanometer (Critikon Dura-cuf, GE Medical Systems, Wisconsin, USA) immediately prior to, and immediately following, the US examination. The subject was as relaxed as possible and was instructed not to talk during the measurement procedure. BP was measured in a supine position from the right and left brachial arteries below the level of the right atrium and the average of all these readings was used for analysis. Subjects whose BP was outside the mean age adjusted normal values were excluded from further evaluation (15).

CT Measurements of Fat

Subjects underwent CT measurements of fat accumulation using a General Electric Hilite Advantage scanner (General Electric Healthcare, Milwaukee, WI) with the same standardized reference phantom for simultaneous calibration and with specially designed software for fat and bone measurements. All scans were obtained by the same CT technologist using the following technical factors: 80 kVp (abdomen) or 120 kVp (femur), 70 mAs, 2 sec, and 10 mm slice thickness. The time required for CT examinations was approximately 10 minutes, and the radiation exposure of this limited CT study was 100–150 mrem (1.0–1.5 mSv).

Measurements of the areas of visceral fat (VF; cm^2) and subcutaneous fat (SF; cm^2) were obtained at the umbilical level. For the purpose of this study, SF was defined as the amount of adipose tissue located between the skin and the rectus muscles of the abdomen, the external oblique muscles, the broadest muscles of the back and the erector muscles of the spine at the level of the umbilicus. VF was defined as the intraabdominal adipose tissue surrounded by the rectus muscles of the abdomen, the external oblique muscles, the lumbar quadrate muscle, the psoas muscles, and the lumbar spine at the same level. Measures of the fat density (FD) of the marrow canal were obtained at the midshaft of the femurs.

CT numbers express the measure of the linear attenuation of the x-ray beam through the medium in that space and are defined as Hounsfield units (HU), using the linear attenuation coefficient of water (HU=0) and air (HU= -1000); using these parameters, HU for fat fall between a range of negative values (16). For the purpose of this study, CT values for FD in HU were converted into density values (g/cm^3) based on previously published studies that calculated CT attenuation values for human tissues, including adipose tissue (17-19). Specifically, HU measures for all subjects were converted into mass density using a function taken from Schneider et al for CT ranges between -98 and 14; density = $(1.018 + 0.893 \times 10^{-3}HU)(g/cm^3)$ (17). It should be stressed that, since marrow is comprised of hematopoietic tissue (+HU) with a density of 1.06 g/cm^3, and fatty tissue (-HU) with a density of 0.92 g/cm^3, the higher the density of marrow tissue the lower the fraction of marrow fat (17). This fraction changes during growth and throughout life in a predictable and orderly age-, bone- and site-specific fashion (20-22). In contrast to the vertebral body and the metaphyseal regions of the long bones, the shaft of the femur contains no trabecular bone. Additionally, by 15 years of age, the marrow in the shaft of the long bones is mostly comprised of fat, and CT values for the density of the marrow at this site mainly reflect the tissue density of fat; although from menarche to menopause females are known to have more hematopoietic tissue in the marrow (23, 24). Hence, to avoid the confounding effects of blood conversion and trabecular bone, we chose to analyze the shaft of the long bones. Intra- and inter-coefficients of variation (CV) for repeated measurements of VF and SF ranged from 1.5% to 3.5% and were 1% for FD (11, 25).

Biochemical Determinations

After an overnight fast, blood samples were obtained. Glucose was analyzed by the Ortho vitros 950 via dry chemistry method and insulin by the Magic Light analyzer via immunochemiluminometric assay that utilizes paired antibodies selectively reactive to intrinsic insulin. Lipid profiles, including measures of triglycerides, total cholesterol and low, very low and high density lipoproteins (LDL, VLDL and HDL respectively), were determined using a direct measurement method for the enzymatic determination of cholesterol esters (26). Cardio-sensitive reactive protein (CRP) was measured by latex-enhanced nephelometry. Using the Homeostasis Model Assessment (HOMA-IR) formula (insulin [mU/l] × glucose [mmol/l]) / 22.5, insulin resistance was calculated (27). The ratio between high-to-low lipoproteins was determined as surrogate of dyslipidemia (LDL +VLDL/HDL).

Statistical Analyses

Student’s t test for unpaired data was used to compare mean values between genders, and simple correlations to investigate the association between the various fat compartments and age, anthropometric parameters, blood pressure, CIMT measures, glucose, insulin, lipids, CRP and bone measures. Multiple linear regressions were performed using the different fat measures as dependent variables, and BMI, the waist-to-hip ratio, and biochemical determinations as independent variables. The StatView® statistical software (SAS Institute Inc. Cary, NC, 27513, USA) was used for these analyses. Quantitative variables are expressed as mean ± standard deviation (SD).

RESULTS

Table 1A shows age, anthropometric measurements, blood pressure, CIMT measures and CT values for fat in all study subjects. BMI ranged from 17.2-36 kg/m^2, reflecting a distribution from normal weight to obesity according to CDC definition. Overall, 19.0% of the subjects were overweight, and 9.9% were obese. As expected, values for height, weight, waist circumference, waist-to-hip ratio, systolic BP and marrow adiposity were significantly greater in males, while those for SF and diastolic PB were greater in females; there were no gender differences in age, BMI, hip circumference, CIMT values or VF. Table 1B summarizes biochemical measures of carbohydrate and lipid metabolism and inflammatory markers; women had higher values for insulin, HOMA-IR and HDL cholesterol, whereas no gender differences were seen for glucose, LDL or VLDL cholesterol, or PCR. Due to higher values for HDL cholesterol, females also exhibited a more favorable lipoprotein ratio.

Regardless of gender, VF and SF were significantly associated with each other (*r*'s= 0.74 for females, 0.78 for males; both *P*'s <0.0001) and were each strongly related to weight, BMI, waist and hip circumferences, insulin, triglycerides and the low to high density lipoprotein ratio. Significant associations were found between VF and CIMT measures in males, but not in females (Table 2). In contrast, marrow adiposity was not related to measures of VF or SF, to any anthropometric parameters (Figure 1), to CIMT values or to any biochemical measures of carbohydrates or lipid metabolism (Tables 2 and 3); this was true for males and females (p=ns for all correlations). No significant associations were found between any of the three fat compartments and values for systolic or diastolic BP (r's between -.20 and .11) or inflammatory profile; although CRP and VF showed a trend towards significance in both genders (both *P*'s = 0.06) (Table 2).

Multivariate regression analyses indicated that both BMI and HOMA-IR independently predicted SF in males, and that BMI and the low-to-high density lipoprotein ratio independently predicted VF in both females and males (Table 3).

DISCUSSION

Ample data indicate that the expansion of body fat has tremendous implications for metabolic health and is strongly associated to dyslipidemia and insulin resistance. However, compared to subcutaneous or abdominal fat depots, the relations between marrow adiposity and metabolic risk factors are largely unknown. In our study, we found that in healthy young males and females, CT measures of marrow adiposity at the midshaft of the femurs were not related to any anthropometric parameters or surrogates of adiposity, such as the waist-to-hip ratio, or to the amount of visceral or subcutaneous fat. Moreover, marrow adiposity was not associated to BP, measures of carotid wall thickness, fasting glucose, fasting insulin, insulin resistance, or lipid profile. To our knowledge, no previous study has assessed the relation between marrow adiposity and CVD risk factors.

In contrast, like others and as expected, we found that visceral and subcutaneous fat compartments were strongly associated with each other and with anthropometric and biochemical measures of metabolic risk. BMI was the main predictor of these two fat

depots, accounting for more than 50% and 70% of the variance of visceral and subcutaneous fat, respectively, regardless of gender. Moreover, HOMA-IR predicted 1.8% of the variance of SF in males, while the low-to-high lipoprotein ratio was predictive of VF in both females and males; 5.7% and 6.0%, respectively.

Our findings are consistent with previous studies in animal models indicating that marrow fat is not affected by insulin or long periods of starvation (12, 28). They are also in agreement with two previous studies in humans showing a lack of association between marrow adiposity and anthropometric or abdominal fat depots measures (29, 30); these investigations used a combination of DXA to measure abdominal adiposity and magnetic resonance (MR) techniques or histology to evaluate marrow fat, while, in the current study, we used CT to analyze this relation. Although no clinical investigations have previously assessed the relationships between marrow adiposity and energy metabolism, there is anecdotal evidence that marrow fat may be metabolically independent of other fat compartments. Indeed, the progressive loss of body weight in anorexia is associated to an increased amount of fat in the marrow, at least until extreme levels of thinness (31). Similarly, acquired lypodystrophy leads to a massive loss of body fat, specifically, of subcutaneous fat in the hips and thighs, and to a lesser degree, of visceral fat, yet marrow adiposity remains preserved (32).

Previous findings by us and others indicate marrow adiposity to be reciprocally related to the amount of bone in both the axial and the appendicular skeleton (11, 30, 33-35). Whether marrow and extraskeletal adipocytes arise from different clonal cells or share a common progenitor that undergoes specific proliferation and differentiation based on location, is yet to be elucidated. In this regard, the superficial and deep subcutaneous fat depots in swine were found to arise from two different progenitors, the perifollicular stromal cells and the mesenchyme, respectively (36).

The use of sophisticated imaging technology, the inclusion of both genders, the evaluations of multiple adiposity and metabolic biochemical measures and the examination of a homogeneous cohort of teenagers and young adults are strengths of this study. The cross-sectional design is a major limitation, and further investigations will be needed to establish why skeletal and extra-skeletal adiposity are not related. Although in the current study we did not assess key determinants of fat accumulation, such as dietary and alcohol intake, since weight, BMI, waist circumference, hip circumference or their ratio were not related to marrow fat, it is unlikely that these variables influenced our results. Additionally, our results are limited to young adults who were not overly obese (BMI >36) and cannot be extrapolated to other age groups or populations. Lastly, while CT determinations of FD have not been validated in the same individuals with histomorphometry, previous in vitro studies have assessed the accuracy of CT measures of marrow adiposity (37).

In conclusion, marrow adiposity in the femurs of healthy teenagers and young adults is not related to visceral or subcutaneous fat depots, nor to metabolic or CVD risk factors. Our findings support the notion that the negative metabolic health outcomes known to be associated to obesity are independent of marrow fat.

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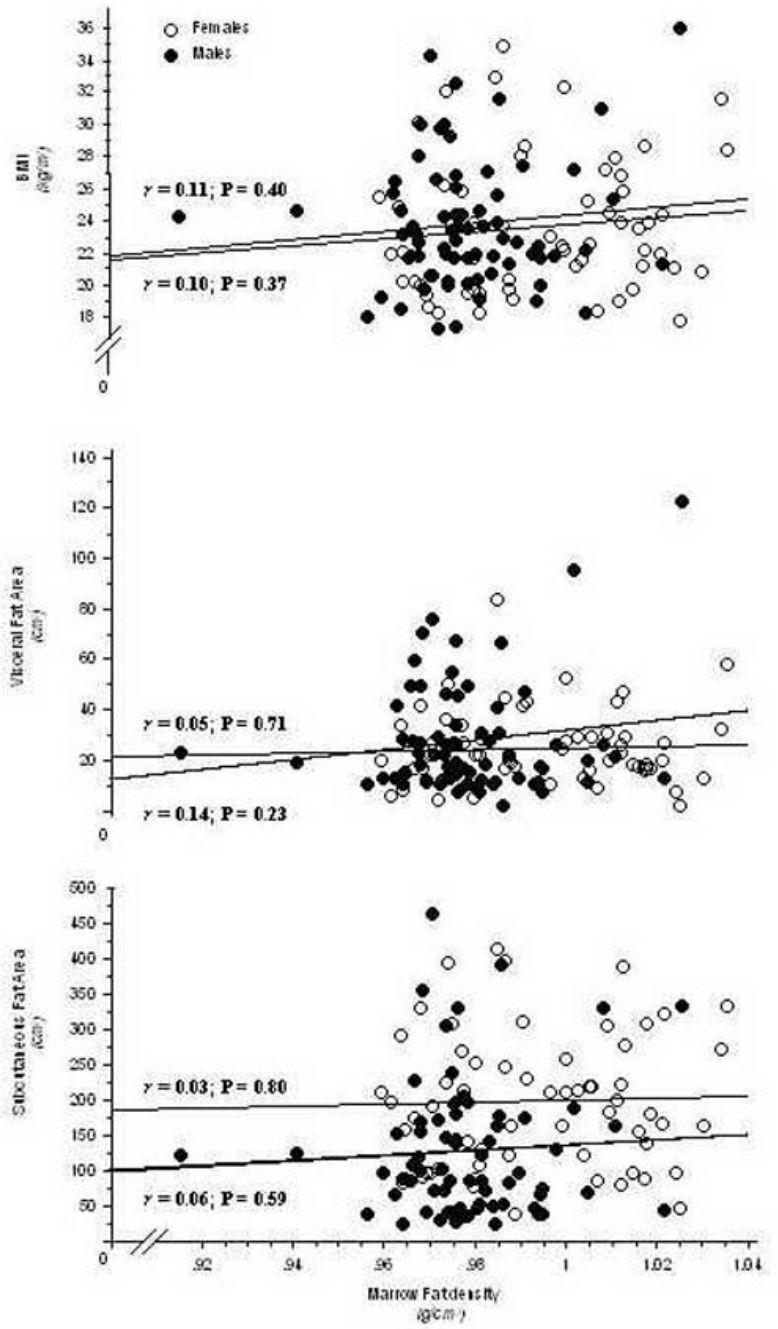

Figure 1.

Correlations between CT values for marrow fat density and measures of visceral and subcutaneous fat areas and BMI in both males and females. Correlations are not statistically significant ($P > 0.05$).

Table 1

A. Age, anthropometric parameters, blood pressure, CIMT and fat compartments measurements in 71 males and 60 females. B. Glucose, insulin and lipid profiles in 71 males and 60 females

	Males	Females
	Means ± SD (range)	**Means ± SD (range)**
Age (years)	19.0 ± 1.8 (16.0 – 24.3)	19.0 ± 2.1 (16.0 – 24.9)
Height (cm) †	172.8 ± 8.1 (152.0 – 190.5)	161.2 ± 6.4 (143.0 – 172.8)
Weight (kg) †	70.9 ± 12.3 (51.0 – 110.0)	61.2 ± 10.9 (45.5 – 92.0)
BMI (kg/m^2)	23.8 ± 3.9 (17.2 – 36.0)	23.6 ± 4.1 (17.9 – 34.8)
Waist circumference (cm)*	81.6 ± 10.6 (63.6 – 114.0)	77.5 ± 9.4 (61.0 – 96.0)
Hip circumference (cm)	94.8 ± 10.7 (56.0 – 121.4)	96.6 ± 7.9 (78.1– 112.0)
Waist/Hip ratio†	0.87 ± 1.0 (0.66 – 1.41)	0.80 ± 0.07 (0.67 – 0.97)
Sistolic BP (mmHg) †	114.1 ± 10.1 (91.5 – 137.0)	107.3 ± 9.0 (89 – 136.5)
Diastolic BP (mmHg)*	57.6 ± 5.6 (44.0–74.0)	60.1 ± 6.5 (46.5 – 78.5)
Mean CIMT (mm)	0.379 ± 0.039 (0.286 – 0.478)	0.385 ± 0.045 (0.292 – 0.508)
CT Subcutaneous Fat (cm^2) †	129.8 ± 95.9 (27.0 – 463.8)	200.1 ± 94.4 (42.2 – 417.3)
CT Visceral Fat (cm^2)	27.7 ± 22.0 (2.6 – 122.5)	24.5 ± 15.0 (2.3 – 84.1)
CT Marrow fat density (g/cm^3) †	0.979 ± 0.017 (0.915 – 1.036)	0.994 ± 0.021 (0.959 – 1.036)

	Males	Females
	Means ± SD (range)	**Means ± SD (range)**
Insulin (mU/l) *	4.0 ± 2.4 (1.0– 13.0)	5.2 ± 3.1 (1.0– 13.0)
Glucose (mg/dl)*	89.2 ± 7.4 (64.0 –103.0)	86.0 ± 5.8 (71.0 –104.0)
HOMA-IR*	0.9 ± 0.6 (0.2 – 3.1)	1.2 ± 0.7 (0.2 – 3.3)
Triglycerides (mg/dl)	85.2 ± 45.1 (19.0 – 234.0)	78.7 ± 34.2 (31.0 – 227.0)
Total Cholesterol (mg/dl)	150.2 ± 29.0 (82.0 – 257.0)	155.5 ± 25.5 (90.0 – 213.0)
HDL cholesterol (mg/dl)*	45.5 ± 10.3 (25.0 – 90.0)	52.4 ± 10.5 (30.0 – 77.0)
LDL cholesterol (mg/dl)	87.7 ± 25.2 (27.0 – 174.0)	87.4 ± 22.3 (24.0 – 146.0)
VLDL cholesterol (mg/dl)	17.0 ± 9.1 (4.0 – 47.0)	15.7 ± 6.8 (6.2 – 45.0)
LDL+VLDL cholesterol (mg/dl)	104.8 ± 28.7 (34.4 – 202.0)	103.2 ± 25.6 (35.2 – 167.0)
Ratio LDL+VLDL/HDL*	2.4 ± 0.9 (0.7 – 5.4)	2.1 ± 0.8 (0.6 – 5.0)
CRP (mg/dl)	2.1 ± 5.2 (0.2 – 30.5)	1.82 ± 2.72 (0.2 – 16.2)

BP blood pressure; CIMT carotid intima media thickness

* $P<0.05$

† $P<0.0001$

* $P<0.05$

Table 2

Simple correlations between fat compartments and anthropometric, CIMT and metabolic measures in 71 males and 60 females

	Fat Compartments					
	Subcutaneous Fat Area (cm^2)		Visceral Fat Area (cm^2)		Marrow Fat Density (g/cm^3)	
	Females	Males	Females	Males	Females	Males
Age	.11	.28*	.14	.48‡	.24	-.07
Weight	.82‡	.81‡	.70‡	.69‡	.03	.02
BMI	.86‡	.85‡	.77‡	.72‡	.12	.11
Waist circumference	.80‡	.87‡	.73‡	.74‡	.01	.04
Hip circumference	.77‡	.69‡	.52‡	.58‡	.14	.05
Waist:Hip ratio	.37†	.23	.51‡	.19	-.12	-.04
Mean IMT (mm)	.17	.23	.15	.24*	.17	.22
Insulin	.35†	.48‡	.33†	.31†	-.01	.04
Glucose	.19	.26*	-.00	.25*	.10	-.14
HOMA	.35†	.48‡	.29*	.32†	.02	.03
Triglycerides	.32*	.44‡	.40†	.51‡	.23	-.02
Cholesterol tot	.22	.22	.18	.37†	.03	-.11
HDL cholesterol	-.41†	-.26*	-.52‡	-.19	-.03	-.09
LDL cholesterol	.35†	.20	.32*	.32†	-.02	-.08
VLDL cholesterol	.32*	.45‡	.40†	.51‡	-.23	-.03
LDL+VLDL/HDL ratio	.52‡	.41†	.62‡	.54‡	.02	.05
CRP	.15	.15	.24	.22	.15	.09

*$P < 0.05$

†$P < 0.01$

‡$P < 0.0001$.

Table 3

Multivariate regression analyses for marrow, visceral and subcutaneous fat prediction

	Females		Males	
	β	P	β	P
Subcutaneous Fat Area				
BMI	19.26	<0.0001	18.23	<0.0001
Waist/hip ratio	-83.53	0.44	-82.48	0.19
HOMA-IR	-4.58	0.66	31.76	0.007
(LDL+VLDL)/HDL	12.17	0.21	11.21	0.15
R^2adjusted	0.73		0.74	
Visceral Fat Area				
BMI	2.11	<0.0001	3.14	<0.0001
Waist/hip ratio	36.90	0.058	-16.83	0.36
HOMA-IR	-2.69	0.14	3.94	0.25
(LDL+VLDL)/HDL	5.50	0.002	7.94	0.0009
R^2adjusted	0.66		0.57	
Marrow Fat Density				
BMI	0.001	0.19	0.001	0.40
Waist/hip ratio	-0.068	0.15	-0.014	0.50
HOMA-IR	-4.546E-4	0.91	2.315E-4	0.93
(LDL+VLDL)/HDL	8.754E-6	0.99	-8.591E-5	0.98
R^2adjusted	-		-	